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Rapid Purification of Recombinant Membrane Proteins in Multiple Detergents

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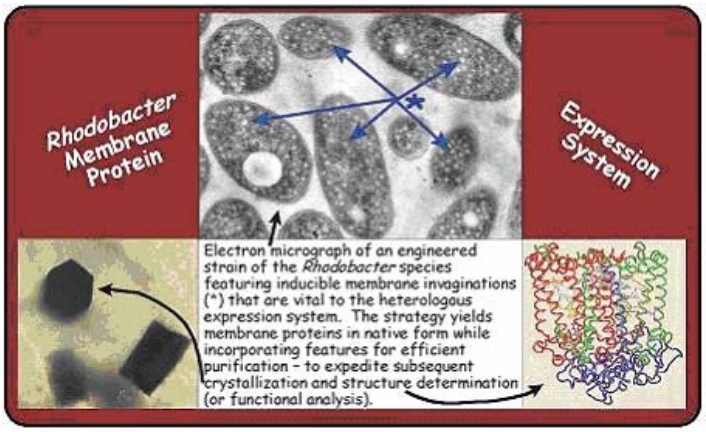
The cell membrane is embedded with proteins that play pivotal roles in the survival and ability of an organism to respond to external stimuli. Our understanding of membrane proteins is limited mainly because, unlike soluble proteins, it is difficult to produce membrane proteins in qualities and quantities that are sufficient for crystallization attempts and functional analyses. Our lab has exploited the membrane physiology of the photosynthetic bacterium *Rhodobacter sphaeroides* and has been successful in transporting a large number (~500) genes encoding poly-histidine tagged membrane proteins from foreign organisms for expression in *R. sphaeroides*. This allowed for membrane-localized production of membrane proteins compatible with this system at levels greater than 1mg/L cell culture for more than half of the target membrane proteins surveyed. The goal of this study was to develop generic methods for solubilizing and purifying these foreign, affinity-tagged proteins from the membranes of *R. sphaeroides* to obtain highly concentrated and pure samples. Here, we report our findings on the use of the detergent Deriphat 160 to solubilize these foreign proteins from *Rhodobacter* membranes in high yield. Subsequently, these membrane proteins are purified in two steps (affinity plus size-exclusion chromatography) using semi-automated scripts running on an ÄKTA-FPLC.

This rapid two-dimensional purification protocol allows for the reproducible isolation and purification of the target protein at quantities (mg scale) sufficient for structural and functional studies. In addition, we discuss general means by which these purified products are exchanged into multiple detergents for later use in crystallization trials



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